106. (New) The kit of claim 46, comprising a paramagnetic particle or bead coated with antibody and a paramagnetic particle or bead not coated with antibody.

107. (New) The kit of claim 78, comprising a paramagnetic particle or bead coated with antibody and a paramagnetic particle or bead not coated with antibody.

REMARKS

Applicants have received and reviewed a final Office Action dated December 23, 1997 in the parent application. By way of response: Claims 22, 23, 28, 29, 33, 34, 36, 37, 39-43, 46-48, 51, 59-62, 64, 66, 71, 72, 74, 75, 78 and 79 have been amended; claims 26, 27, 30-32, 44, 45, 49, 50, 52-58, 63, 65, 68, 70, 76 and 77 have been canceled; and new claims 80 through 107 have been added. Claims 22-25, 28, 29, 33-43, 46-48, 51, 59-62, 64, 66, 67, 69, 71-75 and 78-107 are pending. No new matter has been added by the amended or newly presented claims. Applicants submit the amended and newly presented claims are supported by the specification.

For the reasons given below, Applicants submit that the amended and newly presented claims are in condition for allowance and notification to that effect is earnestly solicited.

Petition for Extension of Time

It is noted that a three-month Petition for Extension of time is necessary to provide for timeliness of the response. A request for such an extension is made extending the time for response from March 23, 1998 to June 23, 1998.

Rejection of Claims Under § 112, Second Paragraph

The Examiner rejected claims 29, 61 and 78-79 under 35 U.S.C. § 112, second paragraph. The Examiner objected to a trademark and certain other terms in the claims.

Claims 29 and 61 no longer include the trademark objected to by the Examiner. Rather, the claims include a generic term, as listed in the Sigma catalog, for the surfactant Tween 20. Amended claim 78 clearly recites the function of each of the antibodies involved.

Accordingly, it is believed that the amended and newly presented claims fully comply with § 112, second paragraph, and withdrawal of this rejection is respectfully requested.

The Prior Art Rejections

The Examiner rejected claims 22-27, 30-33, and 36 under 35 U.S.C. § 102(b) as anticipated by *Widder et al.* (EP 016552). The Examiner rejected claims 28 and 29 and 48-65 under 35 U.S.C. § 103(a) as obvious over *Widder et al.* in view of *Connelly et al.* (U.S. Patent No. 5,422,277). The Examiner rejected claims 34-35, 37-45 and 66-77 under 35 U.S.C. § 103(a) as obvious over *Widder et al.* in view of *Kemmer et al.* and *Holmes et al.* The Examiner rejected claims 46-47 under 35 U.S.C. § 103(a) as obvious over *Widder et al.* in view of *Forrest et al.* Applicants respectfully traverse these rejections.

There are numerous differences between the present invention and the disclosure of the *Widder et al.* reference. For example, on page 7, lines 1-3 in the previous Office Action, the Examiner states the difference between *Widder et al.* and the present invention: «Widder et al. differ from the instant invention in failing to teach the use of enzyme labels and an avidin/biotin binding system. Widder et al. also does not teach using fixatives to pretreat the sample».

Further differences between the present method and *Widder et al.* are:

A. *Widder et al.* have only performed a coarse separation of blood cells, not detection of individual cells.

B. *Widder et al.* have made particles with protein A imbedded. We understand that their particles are not uniform regarding size and amount of protein A on the surface. Thus, when the antibodies bind to protein A one cannot know the amount of antibody pr. particle and the exact orientation of the antibody. These disadvantages do not exist in the present method which therefore could not be anticipated since the present method would not meet the required specifications with those disadvantages.

C. We undestand that the content of iron in *Widder's* particles varies. This is unacceptable for the present method.

Furthermore, due to the A-C the particles of *Widder* will adhere to non-target-cells and target-cells alike and the specificity of the method will be reduced.

D. Protein A will also show specific binding to B-cells and plasma cells, both of which are non-target-cells according to the present method.

E. *Widder's* test system is very simple and the magnetic strength of their particles is not powerful enough to pull a few target-cells out of a population of several million cells, as is the requirement of the present method.

In conclusion regarding the Widder reference alone, if a person with knowledge in the art should think of using monoclonal antibodies on the particles, on which *Widder's* used protein A, he would still believe that the method had the same disadvantages typical for *Widder's* method. We understand that this was the type of criticism Applicants received from his colleagues at the Norwegian Radium Hospital. Furthermore, such disadvantages were demonstrated by *Holmes et al.* by using avidin/biotin regarding blood cells, which resulted in an unspecific binding because normal blood cells also express the target antigen. We thus ask, how could the Examiner or a worker skilled in the art expect that a known method should be used to give up until 100% specificity when all published materials show very unspecific results?

Widder separates cells when only two types are present by using radioactive chromium, while the idea of the present invention is to detect individual target-cells and the detection is performed by looking at the target-cell-rosettes in a microscope. The present inventors have surprisingly succeeded in avoiding the unspecific binding characterizing the other methods. The inventors further use the particle-target-cell complex to visually detect the cells in the microscope. No one has done that previously, because it has not been possible to obtain particle binding exclusively to target cells.

The Examiner is correct in commenting that the use of detergents to treat cells is well known in the art. However, it is also well known in the art that use of detergents does not necessarily increase the specificity of the method. Thus, persons with knowledge in the field would not think that use of detergents (*Connelly*) would make *Widder's* method more specific. The inventors actually tried detergents without obtaining sufficient specificity. Then the combination of detergents in low concentration and low temperatures surprisingly gave the required high specificity, against the advice of their colleages. Nobody had ever thought of this and it was not obvious by combining *Widder* and *Connelly*.

The Examiner asserts that it could be obvious to use antibodies to immobilize antibodies on the surface of magnetic particles. However, the Examiner's assertion requires that THE

METHOD NOT BE MORE SPECIFIC THAN *WIDDER'S*. However, regarding the present method, it was not obvious because the absolute specificity required.

If a person in the field was interested in coarse separation of cells he/she might use the method of *Widder* in view of *Kemmer et al.* and *Holmes et al.*, since both *Kemmer* and *Holmes* are unspecific methods. Thus, a person with knowledge in the field would see absolutely no benefit in combining *Widder* with *Kemmer* and *Holmes* to detect specific target-cells. In such a method non-target-cells will be included in the rosettes and the method would be useless for diagnostic measures. Even though *Kemmer's* method is demonstrated on a solid tumor which is characterized by a great number of malignant target-cells in relation to normal cells, his results were quite unspecific. Seeing this, a person with skill would keep far away from such a method, with the specificity requirements facing the present inventors. In *Holmes et al.* it would have been completely impossible to detect the cells specifically because the number of particles bound to non-target-cells would be the same as the number of particle bound to target-cells, because also subgroups of normal cells will express the target antigen on their surfaces. This is not important regarding purging, however, in a diagnostic method all cells with bound bead rosettes must be target cells. There is, therefore, absolutely no «reasonable expectation of success», rather than opposite, based on *Widder* and *Holmes*.

Accordingly, based on the foregoing differences, it is respectfully submitted that the references cited by the Examiner, either alone or in combination, neither teach nor suggest the claimed methods and kits, and withdrawal of the prior art rejections is respectfully requested.

Summary

In summary, each of claims 22-25, 28, 29, 33-43, 46-48, 51, 59-62, 64, 66, 67, 69, 71-75, and 78-107 are in condition for allowance and notification to that effect is earnestly solicited. The Examiner is invited to telephone Applicants' undersigned representative if the Examiner believes that prosecution of this application will be advanced thereby.

Respectfully submitted,

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